US-PAT-NO: 6080576
DOCUMENT-IDENTIFIER: US 6080576 A
TITLE: Vectors for gene trapping and gene activation

Brief Summary Text - BSTX (10):

The present invention relates to the construction of a novel vector comprising a 3' gene trap cassette that allows for high efficiency 3' gene trapping. The presently described 3' gene trap cassette comprises in operable combination, a promoter region, an exon (optionally characterized by a translation initiation codon and open reading frame and/or internal ribosome entry site), a splice donor sequence, and, optionally, intronic sequences. The splice donor (SD) sequence is operatively positioned such that the exon of the 3' gene trap cassette is spliced to the splice acceptor (SA) site of a downstream exon or a cellularly encoded exon. Typically, the presently described 3' gene trap cassette will trap cellular 3' exons with sufficient efficiency to enable the facile detection, screening, and identification of at least about 10,000 distinct 3' gene trapped cellular exons (generally representing approximately 10,000 different genes--the number may be reduced by the fact that independent integration events can occur within different introns/exons within the same gene), preferably at least about 15,000 distinct 3' gene trapped cellular exons, more preferably at least about 25,000 distinct 3' gene trapped cellular exons, and most preferably at least about 50,000 distinct 3' gene trapped cellular exons.

Detailed Description Text - DETX (12):

The presently described vectors incorporate a novel 3' gene trap cassette that typically allows an order of magnitude more target genes to be trapped and identified by DNA sequence as compared to previous vectors. The presently described 3' gene trap cassette comprises, in operative combination, a promoter region that mediates the expression of an exon, and an operative splice donor (SD) sequence that defines the 3' end of the exon, and which is spliced to a splice acceptor (SA) sequence of a trapped cellular exon located 3' to the integrated 3' gene trap cassette. Optionally, the exon may additionally encode an open reading frame or gene and/or the exon can incorporate a ribosome binding site or internal ribosome entry sequence to facilitate the expression of the open reading frame. In general, such a ribosome binding site is present 5' to the initiation codon of an open reading frame or gene.

Detailed Description Text - DETX (53):

In order to test whether codon usage was responsible for the observed inefficiency in splicing, a puro gene was synthesized that incorporated an optimal mammalian codon usage. However, 3' gene trap cassettes that incorporated the modified puro exon were not efficiently spliced. Another possible reason for inadequate splicing is that the puromycin marker is 700 bp long whereas the average length of a first exon is only about 100 bp. Thus, it further remained possible that placing a selectable marker gene next to a promoter hindered the optimal recognition of the puro exon and splice donor sequence by the splicing machinery.

Detailed Description Text - DETX (63):

Since overexpression is one possible outcome of a gene trap event using the 3' gene trap cassette, it is useful to be able to remove the 3' trap/overexpression component. This can be accomplished by flanking any essential component of the 3' trap cassette (essential components may include the promoter, the exon, the splice donor, the intronic sequence or the entire cassette) with recombinase sites such as those recognized by the flp or cre recombinases. In this way, the addition of the corresponding recombinase in cells or in the organism allows one to reverse or remove overexpression as desired.

Claims Text - CLTX (8):

2) a second exon sequence located 3' from and expressed by said promoter, said second exon being derived from a naturally occuring eukaryotic gene, said second exon not encoding an activity conferring antibiotic resistance and said second exon not being a reporter gene;

Claims Text - CLTX (10):

wherein said vector docs not encode a promoter mediating the expression of said first exon, and wherein said vector does not encode a sequence that mediates the polyadenylation of an mRNA transcript encoded by said second exon sequence and expressed by said first promoter.

Claims Text - CLTX (18):

2) an exon sequence located 3' from and expressed by said second promoters said exon being derived from a naturally occuring, eukaryotic gene, said second exon not encoding an activity conferring antibiotic resistance and said exon not being a reporter gene;

Claims Text - CLTX (24):

(c) an exon sequence located 3' from and expressed by said promoter, said exon being derived from a naturally occuring

eukaryotic gene, said second exon not encoding an activity conferring antibiotic resistance and said exon not being a reporter gene;

Claims Text - CLTX (27):

wherein said promoter, exon and splice donor are present in the vector in between said first and second LTR sequence and in an opposite orientation to said first and second retroviral LTR sequences and wherein said vector does not incorporate a sequence that mediates the polyadenylation of an mRNA transcript expressed by said promoter of element (b) and encoded by said exon of element (c).

09/903/640

US-PAT-NO: 6136566
DOCUMENT-IDENTIFIER: US 6136566 A
TITLE: Indexed library of cells containing genomic modifications and methods of making and utilizing the same

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Brief Summary Text - BSTX (14):

Yet another class of vector contemplated by the present invention is a vector for inserting foreign exons into animal cell transcripts that comprises a selectable marker, a promoter element operatively positioned 5' to the selectable marker, a splice donor site operatively positioned 3' to the selectable marker, and a second exon located upstream from the promoter element that disrupts the splicing or read-through expression of the transcript produced by the promoter element. Typically, the second exon may comprise, in operative combination, splice acceptor and splice donor sequences. Optionally, a polyadenylation site may be incorporated in addition to or in lieu of the splice donor sequence. The second exon may also incorporate a nested set of stop codons in each of the three reading frames. Preferably, such a vector shall not comprise a transcription terminator or polyadenylation site operatively positioned relative to the coding region of the selectable marker, and shall not comprise a splice acceptor site operatively positioned between the promoter element and the initiation codon of said selectable marker.

Detailed Description Text - DETX (61):

VICTR 3 was used to gather a set of gene trap clones. A plasmid containing the VICTR 3 cassette was constructed by conventional cloning techniques and designed to employ the features described above. Namely, the cassette contained a PGK promoter directing transcription of an exon that encodes the puro marker and ends in a canonical splice donor sequence. At the end of the puromycin exon, sequences were added as described that allow for the annealing of two nested PCR and sequencing primers. The vector backbone was based on pBluescript KS+ from Stratagene Corporation.

Claims Text - CLTX (9):

b) treating a second group of cells with a second vector to stably integrate into the genome of said cells, said second vector mediates the splicing of a foreign exon 5' to an exon of a cellular transcript and said second vector comprising

Claims Text - CLTX (13):

an exon comprising a second splice donor site upstream from said promoter and a splice acceptor upstream from said second splice donor site; and

Claims Text - CLTX (50):

d) an exon comprising a second splice donor site upstream from said promoter and a splice acceptor upstream from said second splice donor site; and

US-PAT-NO: 6436707
DOCUMENT-IDENTIFIER: US 6436707 B1
TITLE: Vectors for gene mutagenesis and gene discovery

Brief Summary Text - BSTX (10):

The present invention relates to the construction of novel vectors comprising a 3' gene trap cassette that allows for high efficiency 3' gene trapping. The presently described 3' gene trap cassette comprises in operable combination, a promoter region, an exon (typically characterized by a translation initiation codon and open reading frame and/or internal ribosome entry site), a splice donor sequence, and, optionally, intronic sequences. The splice donor (SD) sequence is operatively positioned such that the exon of the 3' gene trap cassette is spliced to the splice acceptor (SA) site of a downstream exon or a cellularly encoded exon. As such, the described 3' gene trap cassette (or gene trap vector incorporating the same) shall not incorporate a splice acceptor (SA) sequence and a polyadenylation site operatively positioned downstream from the SD sequence of the gene trap cassette. In a preferred embodiment, the exon component of the 3' gene trap cassette, which also serves as a sequence acquisition cassette, will comprise exon sequence and a splice donor sequence derived from genetic material that naturally occurs in an eukaryotic cell.

Detailed Description Text - DETX (32):

Alternatively, the exon of the second 5' gene trap cassette can encode, for example, the thymidine kinase (TK) gene. Using such constructs, FIAU, for example, can be used to select against cells that "splice-around" the first, or "mutagenic," 5' gene trap cassette. Generally, the second 5' gene trap cassettes are incorporated into the vector downstream from the mutagenesis enhancer sequences and upstream from the 3' gene trap cassette. Optionally, one of the two tandem 5' gene trap cassettes can be flanked by suitably oriented recombinase sites that allow the subsequent and specific removal of the 5' gene trap cassette. Using such a strategy, a first 5' gene trap exon (e.g., encoding neo resistance) may be removed using a suitable recombinase activity to effectively "activate" the splicing and expression of the second 5' gene trap cassette which (especially when it encodes a suitable marker/signal activity such as .beta.-gal, green fluorescent protein, etc.) can be used to track the expression of the trapped gene in tissue and in cells and tissue samples using established methods.

Detailed Description Text - DETX (37):

The presently described 3' gene trap cassette comprises, in operative combination, a promoter region that mediates the expression of an exon, and an operative splice donor (SD) sequence that defines the 3' end of the exon. After integration into the target cell chromosome, the transcript expressed by the 3' gene trap promoter is spliced to a splice acceptor (SA) sequence of a trapped cellular exon located downstream of the integrated 3' gene trap cassette. Thus, a fusion transcript is generated comprising the exon of the 3' gene trap cassette and any downstream cellular exons the most 3' of which has a polyadenylation signal.

Detailed Description Text - DETX (88):

Since over expression is one possible outcome of a gene trap event using the 3' gene trap cassette, it could prove useful to be able to remove the 3' trap/over expression component. This can be accomplished by flanking any essential component of the 3' trap cassette (essential components may include the promoter, the exon, the splice donor, the intronic sequence or the entire cassette) with recombinase sites such as those recognized by the flp or cre recombinases. In this way, the addition of the corresponding recombinase in cells or in the organism allows one to conditionally reverse or remove over expression as desired.

Detailed Description Text - DETX (119):

In order to test whether codon usage was responsible for the observed inefficiency in splicing, a puro gene was synthesized that incorporated an optimal mammalian codon usage. However, 3' gene trap cassettes that incorporated the modified puro exon were not efficiently spliced. Another possible reason for inadequate splicing is that the puromycin marker is 700 bp long whereas the average length of a first exon is only about 100 bp. Thus, it further remained possible that placing a selectable marker gene next to a promoter hindered the optimal recognition of the puro exon and splice donor sequence by the splicing machinery.

Claims Text - CLTX (1):

1. A vector comprising: a) a 5' gene trap cassette, comprising in operable combination: 1) a splice acceptor; 2) a first exon sequence located 3' to said splice acceptor, said first exon encoding a marker enabling the identification of a cell expressing said exon; and 3) a polyadenylation sequence defining the 3' end of said first exon; and b) a 3' gene trap cassette located 3' to said polyadenylation sequence comprising in operable combination: 1) a first promoter; 2) a second exon sequence located 3' from and expressed by said promoter, said second exon not encoding an activity conferring antibiotic resistance; and 3) a splice donor sequence defining the 3' region of the exon; and

Claims Text - CLTX (2):

wherein said vector does not encode a promoter mediating the expression of said first exon, and wherein said vector does not encode a sequence that mediates the polyadenylation of an mRNA transcript encoded by said second exon sequence and expressed by said first promoter.

Claims Text - CLTX (4):

3. The vector of claim 1 wherein said second exon and splice donor sequences are the same as or substantially the same as sequences existing in a naturally occurring eukaryotic gene.

Claims Text - CLTX (8):

7. A genetically engineered retroviral vector comprising: a) a marker gene expressed by a first vector encoded promoter; and b) a 3' gene trap cassette comprising in operable combination: 1) a second vector encoded promoter; 2) an exon sequence located 3' from and expressed by said second promoter, said exon not encoding an activity conferring antibiotic resistance; 3) a splice donor sequence defining the 3' region of the exon; and

Claims Text - CLTX (10):

8. A genetically engineered vector comprising: a) a 5' gene trap cassette comprising in operable combination: 1) a splice acceptor; 2) a first exon sequence located 3' to said splice acceptor, said first exon encoding a marker enabling the identification of a cell expressing said exon; and 3) a polyadenylation sequence-defining the 3' end of said first exon; b) a 3' gene trap cassette located 3' to said polyadenylation sequence and comprising in operable combination: 1) a promoter; 2) a second exon sequence located 3' from and expressed by said promoter, said second exon being of non-prokaryotic origin; 3) a splice donor sequence defining the 3' region of the exon; and

Claims Text - CLTX (11):

wherein said vector does not encode a promoter mediating the expression of said first exon, and wherein said vector does not encode a sequence that mediates the polyadenylation of an mRNA transcript encoded by said second exon sequence and expressed by said promoter.

US-PAT-NO: 6361973
DOCUMENT-IDENTIFIER: US 6361973 B1
TITLE: Promoters for expressing genes in a fungal cell

Detailed Description Text - DETX (54):

The present invention also relates to nucleic acid constructs for altering the expression of a gene encoding a polypeptide which is endogenous to a host cell. The constructs may contain the minimal number of components necessary for altering expression of the endogenous gene. In one embodiment, the nucleic acid constructs preferably contain (a) a targeting sequence, (b) a promoter of the present invention, (c) an exon, and (d) a splice-donor site. Upon introduction of the nucleic acid construct into a cell, the construct inserts by homologous recombination into the cellular genome at the endogenous gene site. The targeting sequence directs the integration of elements (a)-(d) into the endogenous gene such that elements (b)-(d) are operably linked to the endogenous gene. In another embodiment, the nucleic acid constructs contain (a) a targeting sequence, (b) a promoter of the present invention, (c) an exon, (d) a splice-donor site, (e) an intron, and (f) a splice-acceptor site, wherein the targeting sequence directs the integration of elements (a)-(f) such that elements (b)-(f) are operably linked to the endogenous gene. However, the constructs may contain additional components such as a selectable marker.

Detailed Description Text - DETX (57):

The constructs further contain one or more exons of the endogenous gene. An exon is defined as a DNA sequence which is copied into RNA and is present in a mature mRNA molecule such that the exon sequence is in-frame with the coding region of the endogenous gene. The exons can, optionally, contain DNA which encodes one or more amino acids and/or partially encodes an amino acid. Alternatively, the exon contains DNA which corresponds to a 5' non-encoding region. Where the exogenous exon or exons encode one or more amino acids and/or a portion of an amino acid, the nucleic acid construct is designed such that, upon transcription and splicing, the reading frame is in-frame with the coding region of the endogenous gene so that the appropriate reading frame of the portion of the mRNA derived from the second exon is unchanged.

Detailed Description Text - DETX (58):

The splice-donor site of the constructs directs the splicing of one exon to another exon. Typically, the first exon lies 5' of the

second exon, and the splice-donor site overlapping and flanking the first exon on its 3' side recognizes a splice-acceptor site flanking the second exon on the 5' side of the second exon. A splice-acceptor site, like a splice-donor site, is a sequence which directs the splicing of one exon to another exon. Acting in conjunction with a splice-donor site, the splicing apparatus uses a splice-acceptor site to effect the removal of an intron.

Detailed Description Text - DETX (59):

The present invention further relates to methods for producing a polypeptide comprising (a) cultivating a homologously recombinant cell, having incorporated therein a new transcription unit comprising a promoter of the present invention, an exon, and/or a splice donor site operably linked to a second exon of an endogenous nucleic acid sequence encoding the polypeptide, under conditions conducive for production of the polypeptide; and (b) recovering the polypeptide. The methods are based on the use of gene activation technology, for example, as described in U.S. Pat. No. 5,641,670.